

CHANGES IN THE EPIDERMAL HISTOLOGY DURING THE  
SLOUGHING CYCLE IN THE RAT SNAKE *PTYAS KORROS*  
SCHLEGEL, WITH CORRELATED OBSERVATIONS  
ON THE THYROID GLAND

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In an initial description of the histological changes in the snake epidermis associated with periodic skin-shedding, Maderson (1965a) established six arbitrary stages to facilitate reference. Stage 1—the resting stage—seen in the post-shedding period, was described as a condition in which a single outer epidermal generation was present above a stratum germinativum. Little if any cell proliferation appeared to occur during this stage, but it was suggested that pronounced changes occurred in the innermost living cells later in the cycle. These layers of cells, which appeared to be present throughout the resting stage, were termed the presumptive lacunar tissue and clear layer. Stages 2 through 5 described the sequential formation and maturation of the component parts of the new inner epidermal generation—*Oberhautchen*,  $\beta$ - and  $\alpha$ -layers. In Stage 6, the pre-shedding condition, the inner generation possessed two or three immature cell layers, which were interpreted as representing presumptive lacunar and clear layer cells. Subsequent studies of other lepidosaurian reptiles (Lillywhite and Maderson, 1968; Maderson, 1966, 1967, 1968; Maderson and Licht, 1967) revealed further details of the structure of the epidermal generation and suggested that the original interpretation (Maderson, 1965a) of the pattern of histogenic activity involved in the laying-down and maturation of the lacunar tissue and clear layer was either incorrect, or applicable only to the species *Elaphe taeninra*.

Sembrat and Drzewicki (1936), Goslar (1958), Lynn (1960, 1970), Maderson (1965b) and Maderson, Chiu and Phillips (1970) have reviewed the data suggesting a relationship between the thyroid gland and periodic skin-shedding in squamate reptiles. Although it is generally held that thyroid hormone stimulates shedding frequency in lizards, while inhibiting it in snakes, there has been no attempt to correlate snake epidermal changes with thyroid gland histology comparable to Eggert's (1935) study of *Lacerta*.

The present study of the epidermis and thyroid gland histology of *Ptyas korros* provides additional data on the histogenesis of the snake epidermal generation, correlates thyroid activity with the described cellular changes, and permits some explanation of the previous experimental results suggesting differences between snakes and lizards in this context.

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## MATERIALS AND METHODS

From September 19th through October 12th, 1966, 44 mature male *P. korros*, Schlegel were purchased in Hong Kong. Some animals were chosen for purchase on the basis of eye texture to ensure representation of all stages of the sloughing cycle (Maderson, 1965a). Following decapitation, the snakes were weighed, and the weight of the abdominal fat determined. A piece of belly skin was fixed in Bouin's fluid and prepared for histological examination as described by Maderson, (1965a).

The thyroid gland was freed from connective tissue, weighed, and fixed in Bouin's fluid for 48 hrs. It was dehydrated in a series of ethyl alcohol, cleared in chloroform, and embedded in 56° C paraffin. Serial sections through the center of the gland were cut at 7  $\mu$ , mounted and stained with hematoxylin and eosin.

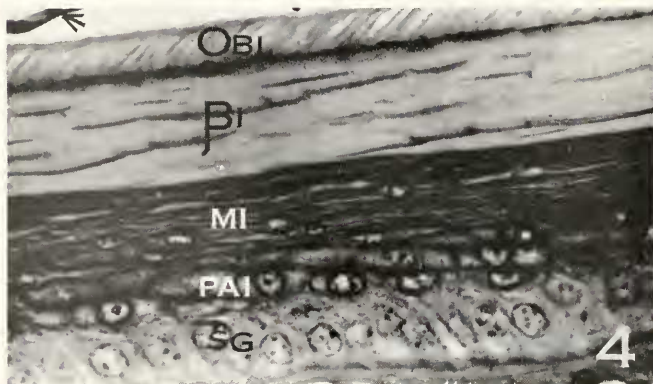
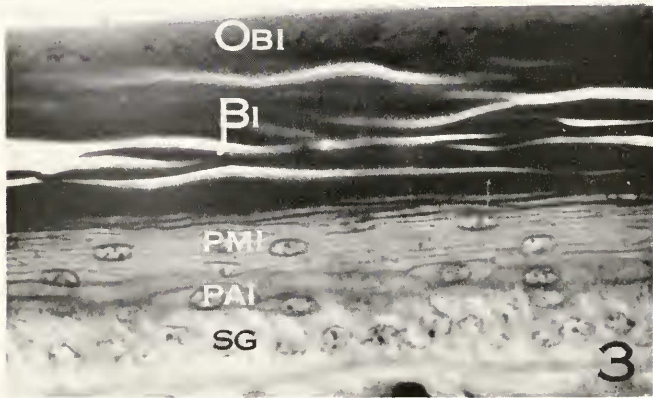
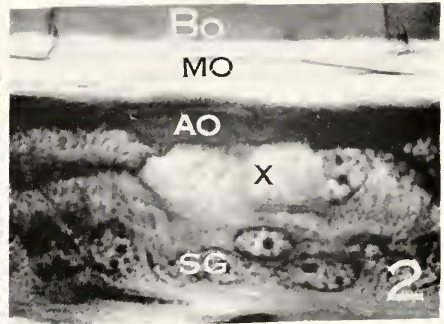
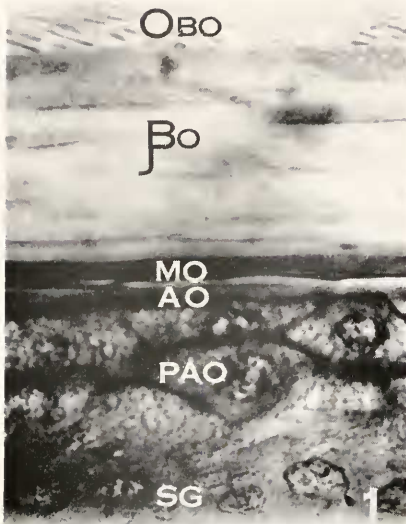
The 44 thyroid glands were divided into 7 groups according to the stage of epidermal development seen in the skin biopsy from the same animal (see Table I). The follicular cell-height for each group was estimated as follows. The 14-31 (mean 20.2) follicles which fell along the longest axis of the gland were examined. The tallest and shortest cell in each follicle was measured with an ocular micrometer. The average of these two values was taken as the cell height for the particular follicle, and the mean of the cell-height for 14-31 (mean 20.2) follicles was taken as the cell height for the gland. The average of the cell heights of all glands examined in any one of the 7 groups was taken as indicating the cell height associated with each of the 7 observable histological conditions of the epidermis.

## RESULTS

*Epidermal histology*

Immediately after shedding, the epidermis of *P. korros* showed an incomplete outer epidermal generation lying above the stratum germinativum (Fig. 1). The generation consisted of a mature *Oberhautchen* (OBO),  $\beta$ -layer ( $\beta$ O) and mesos layer (MO). There was a poorly developed  $\alpha$ -layer (AO) and 3-5 layers of presumptive  $\alpha$ -cells (PAO). This stage of development is referred to in Table I as Stage 0.

A few days later, the epidermis appeared somewhat different (Fig. 2), and showed a condition referred to in Table I as Stage 1. The  $\alpha$ -layer (AO) was then well-developed, and the number of subjacent immature cells reduced by comparison with Stage 0. Stages 2-4 wherein a complete outer epidermal generation (Maderson, 1967) and part of a differentiating inner generation were seen, resembled exactly similar stages described for *Elaphe* (Maderson, 1965a), and these are referred to in Table I. During Stage 5 (Fig. 3), as the new  $\beta$ -cells of the inner generation ( $\beta$ I) finally matured, 8-12 layers of very flattened relatively chromophobic cells were seen just above the columnar, chromophobic cells of the stratum germinativum. These are presumptive mesos cells (see discussion). During Stage 6 (Fig. 4) the  $\beta$ - and mesos cells became completely mature and the nuclei disappeared from them. Presumptive  $\alpha$ -cells (PAI, Fig. 4) appeared and began to mature to form a new  $\alpha$ -layer. At the time of shedding (Stage 6, Table I), the epidermis consisted of a complete, mature outer epidermal generation which would be lost from the body. The inner generation (which soon becomes the



FIGURES 1-4.

functional body surface) consisted of a mature *Oberhautchen*,  $\beta$ - and mesos layers, a partially mature  $\alpha$ -layer and several layers of presumptive  $\alpha$ -cells and exactly resembled the picture described for Stage 0 above.

The animals' external appearance during the cycle changed in a slightly different way from *Elaphe* (Maderson, 1965a). Eye cloudiness began in *P. korros* at Stage 4, reached opacity during Stage 5, and cleared while the epidermis showed a Stage 6 histology.

#### *Thyroid activity (Table I)*

In Table I, thyroid weight in each of the 7 groups is expressed in mg % fat-free body weight. Although some differences are seen, there is no indication that they reflect a cycle of gland activity which can be correlated with the shedding cycle.

Thyroid weight and follicular cell height are not correlated. The cell height was greatest around the time of shedding when the epidermis showed either a Stage 6 condition (9.82  $\mu$ ) or Stage 0 (8.32  $\mu$ ), and lowest during Stage 3 (5.91  $\mu$ ). The follicular cell heights during Stages 1, 2, 3, and 4 were significantly lower than during Stage 0 (about 15–30%,  $P < 0.05$ ). During Stages 3 through 6 there was a steady rise in epithelial cell height (about 45%,  $P < 0.05$ ).

Intracellular colloid was seen in the thyroid follicular epithelium in association with all epidermal conditions except Stages 2 and 3. As shown in Table I, the percentage or the number of animals in each instance showing this condition varied; it was typically found in those animals having thyroids with tall follicular epithelia. While the presence of intracellular colloid probably indicated that active

FIGURE 1. The epidermis of *Ptyas korros* seen one day after sloughing (Stage 0, Table I). There is an incomplete outer epidermal generation above a stratum germinativum (SG). The outer generation has mature  $\beta$ - ( $\beta O$ ) and mesos (MO) layers. There is a poorly developed  $\alpha$ -layer ( $\Delta O$ ) and three layers of presumptive  $\alpha$ -cells (PAO). *Oberhautchen* (OBO). Hematoxylin and eosin.  $\times 850$ .

FIGURE 2. The epidermis of *Ptyas korros* seen during the "perfect resting stage" (Stage 1, Table I). The  $\beta$ -layer ( $\beta O$ ) is out of focus at the top of the picture having separated from the underlying tissues due to splitting of the mesos layer (MO) during histological preparation. The  $\alpha$ -layer ( $\Delta O$ ) is now well developed, and the number of layers of living cells between its base and the stratum germinativum (SG) is reduced by comparison with the condition shown in Figure 1. The cell marked X is probably in the process of final maturation and is the homologue of the "clear cell" shown by Roth and Jones (1967). Hematoxylin and eosin.  $\times 780$ .

FIGURE 3. The epidermis of *Ptyas korros* showing a very early Stage 5 development. Only the inner generation is shown. Note the characteristic splitting of the cells of the  $\beta$ -layer ( $\beta I$ ) in this final stage of maturation (cf. Lillywhite and Maderson, 1968). The mesos cells (PMI) are very flattened units and are quite distinct from the nearly mature  $\beta$ -cells above and first presumptive  $\alpha$ -cells (PAI) beneath. Note the columnar shape, chromophobia and oblique orientation of the germinal cells at this time. *Oberhautchen* (OBI). Hematoxylin and eosin.  $\times 540$ .

FIGURE 4. The epidermis of *Ptyas korros* showing a very early Stage 6 development. Only the inner generation is shown. The  $\beta$ -layer ( $\beta I$ ) is mature and resembles the image shown in Figure 1. The mesos cells (MI) have thickened membranes and the first pycnotic nuclei are seen immediately beneath the  $\beta$ -layer. There are now three layers of presumptive  $\alpha$ - (PAI) cells. When shedding takes place, the inner generation resembles Figure 1 exactly. Hematoxylin and eosin.  $\times 540$ .

colloid synthesis was taking place, the significance of the variation at different epidermal stages is unknown.

Many of the thyroid glands of epidermal stages 6 and 0 were highly vascularized, which suggests that these glands were probably actively secreting.

### DISCUSSION

Since the initial description of the epidermis of *Elaphe* (Maderson, 1965a), further data on the structure of the squamate epidermal generation have become available (Maderson, 1966, 1967, 1968; Maderson and Licht, 1967; Roth and Jones, 1967). The snake mesos layer was indicated by Maderson (1965a), but

TABLE I  
*The weight and the cell-height of the thyroid gland, and the percentage of animals with intracellular colloid at different skin stages during the sloughing cycle in Ptyas*

Skin stages	Body weight @ (g)	No. of snakes	Thyroid gland #		No. of animals with intra- cellular colloid
			Weight mg (% @)	Cell height ( $\mu$ )	
0	223.82 $\pm$ 28.23	11	5.24 $\pm$ 0.51	8.32 $\pm$ 0.40 +	7 (64%)
1	181.20 $\pm$ 17.69	5	6.24 $\pm$ 1.06	6.78 $\pm$ 0.58	1
2	233.67 $\pm$ 18.48	6	4.92 $\pm$ 0.45	6.78 $\pm$ 0.29*	0
3	176.00 $\pm$ 16.00	2	5.45 $\pm$ 0.92	5.91 $\pm$ 0.28	0
4	241.89 $\pm$ 29.32	9	6.30 $\pm$ 0.64	7.17 $\pm$ 0.28	4 (44%)
5	223.33 $\pm$ 33.71	6	5.63 $\pm$ 0.99	6.90 $\pm$ 0.51**	1
6	279.40 $\pm$ 18.07	5	7.10 $\pm$ 1.87	9.82 $\pm$ 1.27	1

# Mean  $\pm$  S.E.

@ Fat-free body weight.

+ Stages 1, 3, and 4 compared with St. 0,  $P < 0.05$ .

\* Stage 2 compared with St. 0,  $P < 0.02$ .

Stage 2 compared with St. 6,  $P < 0.05$ .

\*\* Stage 5 compared with St. 4,  $P =$  not significant.

was assumed to be the outermost part of the  $\alpha$ -layer. Although the relationship of mesos cells to  $\beta$ -cells in terms of protein synthetic capacities is still in doubt, the tissue can now be defined histologically in the snake epidermis. At the onset of Stage 4, presumptive mesos cells are seen as extremely flattened, relatively chromophobic units between the stratum germinativum and the presumptive  $\beta$ -population [Fig. 4 and plate IIa (Maderson, 1965a)]. Their maturation during the latter part of Stage 4 and Stage 5 is accompanied by a chromophobic appearance of the germinal cells [see Fig. 3 in the present study and in Maderson (1965a, pp. 103-106)]. The mesos layer, like the overlying  $\beta$ -layer of the inner epidermal generation is always mature prior to shedding. The mature mesos layer in snake epidermal material is nearly always the site of separation of the  $\beta$ -layer from the subjacent tissues (Maderson and Licht, 1967). The present data from *Ptyas korros* [supported by observations on the cobra (Chiu, Phillips and Maderson, 1969) and

several other ophidian species (Chiu and Maderson, unpublished) compared with the original study of *Elaphe taeniura* (Maderson, 1965a) indicate considerable individual and specific variation in the degree of development of the  $\alpha$ -layer at the time of shedding. As indicated previously (Maderson, 1966) *E. taeniura* is unusual in its lack of variation in this respect. These new data on the mesos layer and the pattern of differentiation of the  $\alpha$ -layer further substantiate the essential morphological similarity of the epidermal systems in the sub-orders of the squamata.

Stage 1 as described here is the homologue of the "one-cell condition" described for *Gekko* (Maderson, 1966, pp. 46-48) and for *Anolis* (Maderson and Licht, 1967, p. 159). It is believed that this "perfect resting condition" exists throughout a period of time in which no, or at least, very little proliferative activity takes place in the stratum germinativum. Evidence from a variety of experimental studies indicates that it is variation in the duration of this period which provides the basis for different inter-slough periods in different species or individuals. It is therefore probable that this is one of, if not the most important, points where the hormonal milieu could control shedding periodicity (Maderson, Chiu and Phillips, 1970).

The present data indicate that in *Ptyas korros* there is a cycle of changes in thyroid epithelial cell height which can be correlated with the changes in epidermal histology associated with periodic skin-shedding. If one accepts the assumption that when the epithelial cells are high, the gland is synthesizing hormone at an enhanced rate and that this hormone is being released into the blood stream, and conversely decreased height indicates relative inactivity of the gland (pp. 129-130, Pickford and Atz, 1957), we can tentatively draw the following conclusion based on the present results. The gland is very active at the time of shedding, activity decreases throughout the subsequent resting phase, reaching a minimum as the first units of the inner epidermal generation become recognizable and then returns to a high level of activity in the latter part of the renewal phase prior to shedding.

The results of his own studies, as well as those of Schaefer (1933), Krockert (1941) and Halberkann (1953; 1954a, b) led Goslar (1964) to propose the following model for the role of thyroid hormone in the control of the snake sloughing cycle. Goslar suggested that although high levels of circulating thyroxine favored germinal proliferation (he eliminated the "perfect resting condition" and obtained  $\alpha$ -hyperplasia by injection with thyroid hormone), generation formation, and subsequent shedding could only occur in the presence of a low level of the hormone in the blood stream. He concluded (Goslar, 1964, p. 4) that the hormone inhibited formation of the "Grenzzone" [the clear layer/*Oberhautchen* complex, the boundary between the outer and inner generations (Maderson, 1967; Maderson and Licht, 1967)]. While this conclusion finds some confirmation in the present results in that the lowest level of thyroid gland activity might be associated with "late" Stage 2-"early" Stage 3 when the clear layer/*Oberhautchen* complex is first recognizable, it may be that the significant factor is the drop in thyroid hormone level during the "late" resting phase. Flaxman, Maderson, Szabo and Roth (1968) showed that generation formation is an intrinsic epidermal capacity dependent on an intraepidermal feed-back mechanism. It has been argued elsewhere (Maderson, Chiu and Phillips, 1970) that this mechanism must be *activated*

during the late resting phase, otherwise there would not be a Stage 2 or 3 morphological condition to identify. To obtain a more dynamic picture of thyroid hormone involvement with shedding in snakes, information on changes in circulating levels throughout the resting phase is needed.

The results of a directly comparable morphological study of *Lacerta* (Eggert, 1935) are quite contrary to the present data. In *Lacerta*, thyroid activity as judged by histological criteria is low immediately prior to shedding and rises to a maximum immediately afterwards. The level of activity remains high throughout the resting phase, the clear layer/*Oberhautchen* complex differentiates while the level is maximal, and gland activity diminishes during Stages 3-5 to rise again at the time of shedding. In *Gekko gecko* (Chiu and Phillips, 1968; Chiu, unpublished data) there is yet another pattern of thyroid gland activity, showing two peaks of activity during one epidermal cycle. Here, the clear layer/*Oberhautchen* complex differentiates in a rising titer of thyroid hormone, there is a subsequent drop around the time of shedding, followed by another peak 7 days after shedding, so that most of the resting phase is associated with low thyroxine levels.

While it is certain that thyroid gland activity does play an important role in controlling shedding frequency in squamate reptiles, it is probable that other endocrine secretions are equally involved. The presently available data do not permit of the establishment of a model pathway system for either snakes or lizards (see discussion, Maderson, Chiu, and Phillips, 1970). There is no direct information regarding the physiological significance of skin-shedding in squamate reptiles, and indeed there is some doubt as to whether it should be regarded as a consummating event in itself or whether it is merely a side effect of other metabolic activities (Maderson, 1965b; Maderson, Chiu and Phillips, 1970). It is of interest to note therefore that there is good evidence for assuming that it is a more tightly controlled process in some taxa than in others (Salthe and Maderson, 1969). This might indicate that the phenomenon of skin-shedding (in company with factors indirectly associated with it and/or directly controlling it) have been evolutionarily selected for more than once, which could account for known differences in hormonal associations in different taxa.

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#### SUMMARY

1. Study of the epidermal histology throughout the sloughing cycle in the snake *Ptyas korros* revealed the existence of a well-defined mesos layer and showed that the  $\alpha$ -layer is completed in the immediate post-shedding resting-phase. These observations supplement previous histological studies of the snake epidermis and further substantiate the essential homology of the epidermal generation in these forms with similar units in lizards.

2. Thyroid weight is not correlated with follicular cell height nor with epidermal cell changes throughout the sloughing cycle.

3. Changes in follicular cell height, indicative of varying levels of gland activity can be correlated with epidermal changes. The shedding complex and the outermost portions of the new inner epidermal generation differentiate during a period of lowest thyroid gland activity; gland activity is highest around sloughing.

4. These results provide a possible explanation for previous experimental studies of thyroid-sloughing relations in snakes. The problem of the difference between snakes and lizards in this context is discussed.

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